Journal of Medical Virology 54:103–106 (1998)

Distinct Prevalence of Antibodies to the E2 Protein of GB Virus C/Hepatitis G Virus in Different Parts of the World

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Since the identification of the new human virus, GB virus C (GBV-C)/hepatitis G virus (HGV), in 1995/1996, reverse transcription polymerase chain reaction remained the sole available diagnostic tool for GBV-C/HGV infection. Recently, a serologic test based on the detection of antibodies to the putative envelope protein 2 (anti-E2) has been introduced. We used this assay for a seroepidemiological survey including 3,314 healthy individuals from different parts of the world, 123 patients from Germany who were suspected to have an increased risk of acquiring GBV-C/HGV infection, 128 multiple organ donors, and 90 GBV-C/HGV RNA positive persons. In European countries, anti-E2 seropositivity ranged from 10.9% (Germany) to 15.3% (Austria). In South Africa (20.3%) and Brazil (19.5%), even higher anti-E2 prevalence rates were recorded. In Asian countries like Bhutan (3.9%), Malaysia (6.3%), and the Philippines (2.7%), anti-E2 positivity was significantly lower. GBV-C/HGV anti-E2 prevalence in potential ''risk groups,'' i.e., patients on hemodialysis and renal transplant recipients, did not vary significantly from anti-E2 seroprevalence in German blood donors. Anti-E2 and GBV-C/HGV RNA were found to be mutually exclusive, confirming the notion that anti-E2 has to be considered as a marker of past infection. ***J. Med. Virol. 54:103–106, 1998.***

KEY WORDS: GBV-C/HGV anti-E2; epidemiological study; viral hepatitis; genome heterogeneity; genotypes

INTRODUCTION

GB virus C (GBV-C)/hepatitis G virus (HGV), is a recently discovered infectious agent distantly related to hepatitis C virus (HCV). Together with GBV-A, GBV-B, and HCV, GBV-C/HGV forms a distinct group in the family Flaviviridae. The GBV-C/HGV genome consists of a positive strand RNA of about 9,400 nucleotides that encodes a polyprotein precursor of approximately 2,900 amino acids [Simons et al., 1995; Linnen et al., 1996; Miyakawa and Mayumi, 1997].

Since the identification of GBV-C/HGV there have been numerous reports on GBV-C/HGV RNA prevalence in different population groups, as well as on a possible association of GBV-C/HGV infection with clinically apparent diseases including acute and chronic non-A–E hepatitis [Linnen et al., 1996; Fiordalisi et al., 1996], fulminant hepatic failure [Yoshiba et al., 1995; Heringlake et al., 1996], aplastic anemia preceded by a GBV-C/HGV positive hepatitis [Byrnes et al., 1996], a specific cholestatic liver disease [Colombatto et al., 1996], and severe cholestatic courses after orthotopic liver transplantation [Ross et al., 1997]. In none of these cases, however, a causative role of GBV-C/HGV could be established. Therefore, the full medical significance of GBV-C/HGV still remains to be elucidated. Epidemiological studies, which would be helpful in uncovering the short and long-term consequences of GBV-C/HGV infections, have been hampered to a considerable extent by the lack of effective serologic

Contract grant sponsor: IFORES (Scientific Foundation of the University of Essen); Contract grant sponsor: DAAD, CAPES.

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Accepted 2 October 1997

tests. Diagnosis up to now had to rely entirely on the detection of the viral RNA by reverse transcription polymerase chain reaction (RT-PCR). Recently, however, a serologic assay for the identification of antibodies to the putative envelope 2 protein (anti-E2) of GBV-C/HGV has been described independently by different research groups [Tacke et al., 1997 a,b; Dille et al., 1997]. We used this antibody test as a convenient tool for an epidemiological survey on GBV-C/HGV seroprevalence including 3,314 healthy individuals from different parts of the world, 123 patients from Germany, who were at a potential risk of acquiring GBV-C/HGV infection, 128 multiple organ donors, and 90 GBV-C/HGV RNA positive persons.

MATERIALS AND METHODS

Samples

A total of 3,314 samples from healthy individuals of both sexes in nine countries were screened for the presence of antibodies to the E2 protein of GBV-C/HGV. Sera were obtained from healthy blood donors in Austria (n = 1,147), Germany (n = 368), Moldowa (n = 125), Spain (n = 400), South Africa (n = 385), Brazil (n = 174), Malaysia (n = 286), and the Philippines (n = 150). Blood samples from Bhutan were drawn from 279 healthy adults who had been enrolled in a seroepidemiological study to investigate the endemic status of hepatitis in the Bhutanese general population [Da Villa et al., 1997]. One hundred and twenty-three serum samples from German patients at a potential risk of acquiring GBV-C/HGV as a result of blood-borne infection were tested for both anti-E2 and GBV-C/HGV RNA. These "risk groups" comprised 31 individuals on maintenance hemodialysis and 92 renal transplant recipients. Additionally, 128 multiple organ donors and 90 GBV-C/HGV RNA positive individuals were included.

Detection of Anti-E2

Antibodies to the E2 protein of GBV-C/HGV were determined by the enzyme immunoassay "μPlate Anti-HG_{env}" (Boehringer, Mannheim, Germany). Viral E2 protein used in this assay was expressed within Chinese ovary hamster cells and was bound to a streptavidin-coated microtiter plate via a monoclonal mouse GBV-C/HGV E2 antibody [Tacke et al., 1997 b]. Calculation of the cut-off value was performed according to the manufacturer's recommendations ($A_{cut\text{-}off} = 0.2 \times A_{Positive\ Control} + A_{Negative\ Control}$; A: absorbance). Samples with borderline absorbances ±15% of the cut-off value were subjected to a second run, where the E2 antigen was omitted to exclude unspecific binding. A specimen was considered negative for anti-E2 whenever $A_{sample\ with\ E2\ antigen}/A_{sample\ without\ E2\ antigen}$ was less than 1.5.

Detection of GBV-C/HGV RNA

GBV-C/HGV RNA was detected by RT-PCR as described elsewhere [Khudyakov, et al. 1997; Viazov et al., 1997] with few modifications. RNA was extracted from 140 μL of serum with a QIAamp viral RNA kit (QUIAGEN, Hilden, Germany) and resuspended in 50 μL of H_2O. Ten microliters of this RNA preparation was transcribed to cDNA by reverse transcriptase (Life Technologies, Gaithersburg, MD) with GBV-C primer YK-877. For nested PCR, the following oligonucleotide primers corresponding to the NS5 region of GBV-C/HGV were used: YK-877: 5′-ACC GAC ACC TTA GAT CCC CAG CCC-3′, YK-874: 5′-CTG ATG TTG CTA GCC TGT GTG AGA-3′, YK-1183: 5′-CAG AAC CAT ACA GCC TAT TGT GAC-3′, and YK-876: 5′-CCT TAC AGT CCT TAT TGC TTC CTC-3′. PCR amplification was performed in a total of 50 μL containing 5 μL of the respective cDNA mixture and 3 units of Taq polymerase (Promega, Madison, WI). In the first round of PCR, primers YK-877 and YK-874 and in the second round primers YK-1183 and YK-876 were used, respectively. Target sequence amplification was done in both rounds for 35 cycles, each consisting of 1 minute at 94°C, 1 minute at 60°C, and 2 minutes at 72°C. An elongation step at 72°C for 7 minutes was added to both rounds of PCR. Amplified DNA was fractionated by 1% agarose gel electrophoresis and the 402-base pair amplification product was visualized by UV fluorescence after staining with ethidium bromide.

TABLE I. Prevalence of Antibodies to the E2 Protein of GBV-C/HGV in Healthy Individuals From Different Parts of the World

Region/country	Samples (n)	Anti-E2 positive (n)	Anti-E2 positive (%)	Confidence limits (95%)
Europe				
Austria	1,147	175	15.3	13.3–17.5
Germany	368	40	10.9	8.0–14.8
Moldowa	125	17	13.6	8.2–20.9
Spain	400	48	12.0	9.0–15.6
Africa				
South Africa	385	78	20.3	16.6–24.9
America				
Brazil	174	34	19.5	14.0–26.3
Asia				
Bhutan	279	11	3.9	2.0–7.0
Malaysia	286	18	6.3	3.8–9.8
Philippines	150	4	2.7	0.7–6.7

Statistical Analysis

Data were explored using 95% confidence limits and a two-sided χ^2 test. Differences were considered to be significant at the 5% level.

RESULTS

The seroprevalence of antibodies to the E2 protein of GBV-C/HGV in healthy individuals from different parts of the world is shown in Table I. In the European countries, anti-E2 seropositivity varied insignificantly from 10.9% (Germany) to 15.3% (Austria). In South Africa (20.3%) and Brazil (19.5%), even higher anti-E2 prevalence rates than in Europe were recorded. In Bhutan (3.9%), Malaysia (6.3%), and in the Philippines (2.7%), respectively, anti-E2 positivity was signifi-

TABLE II. Prevalence of Antibodies to the E2 Protein of GBV-C/HGV in Different Patient Groups

	Hemodialysis	Renal transplant	Organ donors	GBV-C/HGV RNA positive
Samples (n)	31	92	128	90
RNA positive				
n	3	14	7	90
%	9.7	15.2	5.5	100.0
Confidence limits (95%)	2.0–25.8	8.6–24.2	2.1–11.4	96.0–100.0
Anti-E2 positive				
n	4	10	15	1
%	12.9	10.9	11.7	1.1
Confidence limits (95%)	3.6–29.8	5.3–19.1	7.1–18.6	0.03–5.5
Anti-E2 and RNA positive				
n	0	0	0	1
%	0	0	0	1.1
Confidence limits (95%)	0.0–11.2	0.0–3.9	0.0–3.2	0.03–6.1

cantly lower than in the European, African, and American countries investigated here.

Data for the prevalence of GBV-C/HGV markers in German patients, who were at potential risk of acquiring GBV-C/HGV as blood-borne infection, are given in Table II. Percentages of anti-E2 positivity are 10.9 in renal transplant recipients and 12.9 in patients on hemodialysis. None of these two groups demonstrated any statistically significant difference when compared to the anti-E2 seroprevalence in German blood donors (10.9%, Table I). Prevalence of viral RNA was 9.7% in hemodialysis patients and 15.2% in renal transplant recipients. In multiple organ donors, viral RNA was detected in 5.5% and anti-E2 in 11.7%. Not a single individual from the risk and multiple organ donor groups simultaneously tested positive for GBV-C/HGV RNA and anti-E2 viral antibodies. Only 1 of 90 GBV-C/HGV RNA positive subjects had E2 antibodies.

DISCUSSION

We studied the prevalence of antibodies to GBV-C/HGV E2 protein in different parts of the world and in patient groups considered to be at an increased risk for acquiring GBV-C/HGV infection. Our results demonstrate that anti-E2 prevalence rates in healthy blood donors from European, African, and American countries range from 10.9% (Germany) to 20.3% (South Africa) and are higher than the rates reported previously for blood donors from Germany (9.0%), North America (3.0–8.1%), and the United Kingdom (3.3%), respectively [Tacke et al., 1997a; Dille et al., 1997; Karayiannis et al., 1997]. Noteworthy, anti-E2 prevalence in the Asian countries investigated here is significantly lower. There are several possible explanations for this phenomenon: i) low anti-E2 prevalence might indicate a generally low rate of GBV-C/HGV infection in the Asian population, reflecting infrequent exposure to the virus, ii) GBV-C/HGV is insufficiently cleared by infected patients in Asia, and iii) anti-E2 mounted in the infected Asian individuals might escape from detection by the immunoassay employed in this study due to GBV-C/HGV genetic heterogeneity, suggesting that isolates from Asia constitute a distinct genotype or subtype [Muerhoff et al., 1996; Okamoto et al., 1997]. At present, it is difficult to discriminate between these possibilities and investigations to further elucidate this issue are currently in progress.

The absence of GBV-C/HGV RNA in anti-E2 positive individuals and the very rare presence of anti-E2 antibodies in GBV-C/HGV RNA positive patients investigated in this study further sustain the initial findings on the anti-E2 assay, namely, anti-E2 antibodies and GBV-C/HGV RNA are mutually exclusive [Tacke et al., 1997a; Dille et al., 1997]. Anti-E2, therefore, must be considered as a serologic marker of past infection. Our results on the almost identical prevalence of anti-E2 in blood donors, multiple organ donors, hemodialysis patients, and renal transplant recipients at first sight suggest similar rates of exposure to GBV-C/HGV in all groups investigated. On the other hand, percentages of GBV-C/HGV RNA positivity are higher in patients on maintenance hemodialysis and in renal graft recipients than in multiple organ donors. One possible explanation of this finding is that resolution of GBV-C/HGV infection might be a relatively rare event in patients with renal failure characterized by an impairment of their immune response or in immunosuppressed renal graft recipients [Masuko et al., 1996; Neilson et al., 1996]. In nonimmunocompromised individuals, however, rates of chronic GBV-C/HGV infection seem to be much lower [Wang et al., 1996; Alter et al., 1997]. This notion is supported by both our data on high anti-E2 and low GBV-C/HGV RNA prevalence in German blood and organ donors, as well as by previous reports that GBV-C/HGV RNA can only be detected in 1.3–4.7% of healthy German blood donors [Heringlake et al., 1996; Roth et al., 1997].

Taken together, our results on GBV-C/HGV anti-E2 seroprevalence demonstrate a world-wide high rate of GBV-C/HGV infection. Regarding these high infection rates, there should be a natural anxiety in the medical community about the clinical significance of GBV-C/HGV. Therefore, at present, it seems premature to declare GBV-C/HGV an entirely innocent virus, since the welfare of many individuals may be at stake [Miyakawa and Mayumi, 1997].

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